IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:			
Jean-Marie BUERSTEDDE *et al.*		Art Unit:	1633
Appln. No.:	10/590,211	Examiner:	Fereydoun Sajjadi
Filed:	August 22, 2006	Confirmation No.:	5528
For:	Methods for Genetic Diversification in Gene Conversion-Active Cells	Atty. Docket:	P30753US00/21027.002

Declaration of Prof. Dr. Jürgen Wienands Pursuant to 37 CFR §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Prof. Dr. Jürgen Wienands, Ph.D. declare and state as follows:

1. I am currently a professor of cellular and molecular immunology and the head of the Department of Cellular & Molecular Immunology at the Göttingen University, Faculty of Medicine, where I operate a laboratory that studies physiology and patho-physiology of B cells with genetic and biochemical methods including mouse and cell culture methods. I have also authored or co-authored numerous articles in this field. A copy of my professional CV is attached hereto as Exhibit A.

2. I reviewed U.S. Patent Application No. 10/590,211, entitled "Methods for genetic diversification in gene conversion-active cells" and published as WO 2005/080552.

3. I was asked by the Applicant's representative to comment on the questions raised by the Examiner during the prosecution of U.S. 10/590,211.

4. In the present application, a reciprocal relationship between gene conversion and hypermutation in gene conversion- active cells was established. *See* WO 2005/080552, page 9, lines 6-11. This means that if there are no gene conversion donors in the immunoglobulin locus of such a cell, the locus becomes hypermutation active.

5. Gene conversion functions only between highly homologous sequences, such as the pseudo-V genes (gene conversion donors) and the V gene (gene conversion recipient) in the case of the immunoglobulin locus. Hypermutation, also called somatic hypermutation, as a process of highly frequent mutagenesis that takes place in the immunoglobulin locus of higher eukaryotes and ranges between 10^{-5} to 10^{-3} per bp per generation. In contrast, the rate of spontaneous mutation in the mouse and human is estimated to be about 10^{-8} per bp per generation. *See* Drake *et al.*, Rates of Spontaneous Mutation, (1998) *Genetics* 148:1667-1687. Somatic hypermutation is thus not a somewhat increased spontaneous mutation rate, but a different phenomenon.

6. In light of the specification, hypermutation in a cell capable of gene conversion can be obtained by inserting a target nucleic acid into the immunoglobulin locus of a gene conversion-active cell. *See* WO 2005/080552, page 11, lines 19-26, original claim 29.

7. In this situation, the transgene (foreign sequence) becomes a potential recipient of gene conversion events. However, as the transgene does not have any gene conversion donors in the locus (because it is not homologous to the endogenous gene conversion donors, the pseudo-V genes), it gets diversified not by gene conversion, but by hypermutation.

8. For a transgene in an otherwise unmodified immunoglobulin locus, there are no adjacent donor sequences. Therefore, for the transgene, any gene conversion donors are effectively removed due to their lack of homology with the transgene. Accordingly, it is not necessary to actually remove the endogenous pseudo-V-genes to

have hypermutation of the transgene, as the endogenous pseudo-V-genes are no longer gene conversion donors for the transgene.

9. Hypermutation also occurs in the situation where endogenous pseudo-V genes (gene conversion donors) are removed and the endogenous V-gene no longer has homologous sequence for gene conversion to occur.

10. One example of a lymphoma cell line capable of gene conversion is DT40, a cell line derived from a chicken bursal B cell. This cell line is *a priory* gene conversion active; no hypermutation takes place therein. Only upon insertion of a transgene will it "switch" from gene conversion of the V-gene to hypermutating the transgene.

11. In Example 2 of the present application, it is described that new fluorescence proteins can be produced by inserting a green fluorescent protein (GFP) gene, *i.e.* the transgene, into the immunoglobulin locus of a DT40 cell using the vector depicted in Figure 7A. *See* WO 2005/080552, page 19, line 25 - page 20, line 12. The insertion of GFP in this way does not lead to the deletion of the endogenous pseudo-V genes. In the immunoglobulin locus of the genetically modified DT40 cell, GFP becomes diversified by hypermutation, leading to new proteins with variations in color, intensity and half-life of fluorescence. *See* WO 2005/080552, Figure 7B.

12. The system described in Example 2 is also reported in Arakawa *et al.*, *Nucleic Acids Research* 36(1): e1, 2008. As described on pages 3 to 5, the knock-out vector, pHypermut1-eGFP, was used to insert a GFP expression construct into the immunoglobulin light chain locus of DT40 (IgL^{eGFP1}), see Figure 1A. As can be seen from the figure, the part of the IgL locus containing the pseudo-V genes is thereby not removed. The authors reported that the GFP expression construct hypermutated at a high rate (page 3, right-hang column, second section). The fact that there is no need for deleting pseudo-V genes is further confirmed in Blagodatski *et al.*, *PLOS Genetics*, 2009.

13. Finally, Activation-Induced Cytidine Deaminase (AID) is a factor that regulates both gene conversion and hypermutation in the immunoglobulin locus. AID is constitutively expressed in lymphoid cells performing gene conversion or hypermutation. This finding was published by Arakawa *et al.*, *PLOS Biology*, 2004.

I hereby state that all statements made herein based on my own personal knowledge are true and correct and that all statements based on my information and belief are true and correct to the best of my knowledge, and further that all of these statements have been made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issued from this application.

October 25th, 2010
Date

Prof. Dr. Jürgen Wienands

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Education

Institution/ Place	Degree	Period	Field
Kreisgymnasium Heinsberg	Abitur	1981	
University of Köln	Diploma in Biology	1982-1989	Biology
Max-Planck-Institute & Albert-Ludwigs-University of Freiburg	Dr. rer. nat.	1989-1992	Immunology/ Biochemistry
Albert-Ludwigs-University of Freiburg	Habilitation	2001	Molecular Immunology & Biochemistry

Appointments and Honours:

Academic Appointments and Employment

since 08/2004 Full Professor (W3) and Head, Department of Cellular & Molecular Immunology, University of Göttingen

2001 – 2004 Professor (C4) for Molecular Immunology and Biochemistry, University of Bielefeld

1996 – 2001 Group leader (C1) at the Institute for Biology III, University of Freiburg

1994 – 1996 Postdoctoral fellow at the Max-Planck-Institute for Immunobiology, Freiburg i.Br.

1992 – 1994 Postdoctoral fellow, Preclinical Research-Institute Sandoz, Basel, Switzerland

1989 – 1992 Ph.D. project at the Max-Planck-Institute for Immunobiology, Freiburg i.Br.

Other Professional Affiliations and Activities

Since 01/2006 Chair, MD/PhD program *Jacob-Henle-Programm,* Georg-August-University Göttingen

Since 06/2006 Elected full member of the advisory board of the German Government, Commission on Biosafety in Germany, Berlin
Zentrale Kommission für Biologische Sicherheit, ZKBS

04/2007 – 04/2009 Member, *Habilitations* Committee at the Medical Faculty, Georg-August-University Göttingen

04/2007 – 04/2009 Member, Central steering committee for teaching, Georg-August-University Göttingen

Since 04/2009	Member, Steering committee for research at the Medical Faculty, Georg-August-University Göttingen
Since 2010	Member, PhD program *„GAUSS"*, Georg-August-University Göttingen
Membership (Professional Societies)	Advisory Board of the Signal Transduction Society (2003-2009) Advisory Board of the German Society for Immunology (since 2009)
Reviewer (Journals):	Nature Immunology Immunity Blood Journal of Experimental Medicine European Journal of Immunology
Reviewer (Grants):	German Research Foundation (DFG) Research Committee, Anniversary Fund of the Austrian National Bank Boehringer Ingelheim Fonds Internal Research Support, German universities, e.g. Bonn, Heidelberg

Publikationsliste

1990

1. Wienands, J., Hombach, J., Radbruch, A., Riesterer, C. and Reth, M. *Molecular components of the B cell antigen receptor complex of class IgD differ partly from those of IgM.* **EMBO J.** 9:449-455.

2. Justement, L.B., Wienands, J., Hombach, J., Reth, M. and Cambier J.C. *Membrane IgM and IgD molecules fail to transduce Ca^{2+} mobilizing signals when expressed on differentiated B lineage cells.* **J. Immunol.** 144:3272-3280.

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3. Reth, M., Hombach, J., Wienands, J., Campbell, K.S., Chien, N., Justement, L.B. and Cambier, J.C. *The B-cell antigen receptor complex.* **Immunol. Today** 12:196-201.

4. Wienands, J. and Reth, M. *The B cell antigen receptor of class IgD can be expressed on the cell surface in two different forms.* **Eur. J. Immunol.** 21(10):2373-2378.

5. Reth, M., Wienands, J., Tsubata, T. and Hombach, J. *Identification of components of the B cell antigen receptor complex.* **Adv. Exp. Med. Biol.** 292:207-214.

1992

6. Wienands, J. and Reth. M. *Glycosyl-phosphatidylinositol linkage as a mechanism for cell-surface expression of immunoglobulin D.* **Nature** 356:246-248.

1993

7. Reth, M., Hombach J., Weiser, P. and Wienands, J. *Structure and signaling function of B cell antigen receptors of different classes.* **Molecular Mechanisms of Immunological Self-recognition**; Alt, F.W. and Vogel, H. J. (Eds.); Academic Press, Inc., Harcourt Brace Jovanovich, Publishers, p. 69-74.

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8. Baumann, G., Maier, D., Freuler, F., Tschopp, C., Baudisch, K. and Wienands, J. *In vitro characterization of major ligands for Src homology 2 domains derived from protein tyrosine kinases, from the adaptor protein SHC and from GTPase-activating protein in Ramos B cells.* **Eur. J. Immunol.** 24:1799-1807.

9. von Bonin, A., Wienands, J., Manning, U., Zuber, J.F. and Baumann, G. *The βD-sheet residues of the Lck-derived SH2 domain determine specificity of the interaction with tyrosine-phosphorylated ligands in Ramos B cells.* **J. Biol. Chem.** 269:33035-33041.

1995

10. Wienands, J., Freuler, F. and Baumann, G. *Tyrosine-phosphorylated forms of Igβ, CD22, TCRζ and HOSS are major ligands for tandem SH2 domains of Syk.* **Int. Immunol.** 7:1701-1708.

1996

11. Wienands, J., Larbolette, O. and Reth, M. *Evidence for a preformed transducer complex organized by the B cell antigen receptor.* **Proc. Natl. Acad. Sci. USA** 93:7865-7870.

1997

12. Reth, M. and Wienands, J. *Initiation and processing of signals from the B cell antigen receptor.* **Annu. Rev. Immunol.** 15:453-479.

1998

13. Wienands, J., Schweikert, J., Wollscheid, B., Jumaa, H., Nielsen, P.J. and Reth, M. *SLP-65: A new signaling component in B lymphocytes which requires expression of the antigen receptor for phosphorylation.* **J. Exp. Med.** 188:791-795.

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16. Wollscheid, B., Wienands, J. and Reth, M. *The adaptor protein SLP-65/BLNK controls the calcium response in activated B cells.* **Curr. Top. Microbiol. Immunol.** 246:283-289.

17. Wollscheid, B., Reth, M. and Wienands, J. *Characterization of the B cell-specific adaptor SLP-65 and other protein tyrosine kinase substrates by two-dimensional gel electrophoresis.* **Immunol. Lett.** 68:95-99.

18. Yohannan, J., Wienands, J., Coggeshall, M.K. and Justement, L.B. *Analysis of tyrosine phosphorylation-dependent interactions between stimulatory effector proteins and the B cell co-receptor CD22.* **J. Biol. Chem.** 274:18769-18776.

19. Reth, M. and Wienands, J. *The maintenance and the activation signal of the B-cell antigen receptor.* **Cold Spring Harb. Symp. Quant. Biol.** 64: 323-328.

20. Su, Y.W., Zhang, Y., Schweikert, J., Koretzky, G.A., Reth, M. and Wienands, J. *Interaction of SLP adaptors with the SH2 domain of Tec family kinases.* **Eur. J. Immunol.** 29:3702-3711.

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23. Wienands, J. *Signal transduction elements of the B cell antigen receptor and their role in immunodeficiencies.* **Immunobiology** 202:120-133.

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2006

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43. Grabbe, A. and Wienands, J. *Human SLP-65 isoforms contribute differently to activation and apoptosis of B lymphocytes.* **Blood** 108:3761-3768.

2007

44. Stork, B., Neumann, K., Goldbeck, I., Alers, S., Kähne, T., Naumann, M. Engelke, M. and Wienands, J. *Subcellular localization of Grb2 by the adaptor protein Dok-3 restricts the intensity of Ca^{2+} signaling in B cells.* **EMBO J.** 26:1140-1149.

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2009

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2010

55. Rieke, C., Kähne, T., Schweitzer, K., Schraven, B., Wienands, J., Engelke, M. and Naumann, M. *Non-T cell activation linker regulates ERK activation in Helicobacter pylori-infected epithelial cells.* **Cell Signal.** 22(3): 395-403.

56. Borgerding, A., Hasenkamp, J., Engelke, M., Burkhart, N., Trümper, L., Wienands, J. and Glass, B. *B-lymphoma cells escape rituximab-triggered elimination by NK cells through increased HLA class I expression.* **Exp. Hematol.** 38(3): 213-221.